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CHEMOTHERAPY WITH THE SULFONAMIDE DERIVATIVES: GENERAL PRINCIPLES*

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FOLLOWING the introduction of the sulfonamide group of drugs as bacterial chemotherapeutic agents and their first reported clinical application by Foerster[1] in 1933, the use of these compounds in the treatment of infections in man very soon became so apparently successful that they were already widely used clinically before much was known concerning the scope and nature of their chemotherapeutic properties in experimental infections in animals, their mechanism of action, their toxicity, and the principles governing their absorption, distribution, conjugation and excretion. Numerous compounds appeared in rapid succession under a variety of confusing names with conflicting claims concerning their relative merits in this or that type of infection. Gradually, however, careful laboratory investigations began to parallel and influence clinical application, until latterly they have begun quite properly to precede the introduction of new compounds, prepared with the purpose of extending the usefulness of the sulfonamide derivatives in human disease.

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Since the intelligent use of these drugs must be based upon an adequate understanding of their scope and mode of action and their pharmacological properties, it is my purpose in this general discussion to review the development of knowledge concerning these problems. I shall endeavor to point out their bearing on clinical therapeutics. Of necessity I must limit myself to the high-lights since the time available will not permit me to fill in much in the way of detail.

The sulfonamide group of drugs center around para-amino-benzene sulfonamide, first synthesized by Gelmo[2] in 1908. Though not the first compound introduced into chemotherapy, it has become the hub of the wheel and now bears the medical designation, sulfanilamide. For the purposes of the present discussion its derivatives may be conveniently placed in two groups.

The first group, which includes the original prontosil used by Domagk,[3] consists of those derivatives in which substitutions have been made in the para-amino group. Of these the more important are the azo dyes prontosil (Mietzsch and Klarer,[4] 1932; Domagk,[3] 1935) and the more soluble neoprontosil (Domagk,[5] 1935), and benzyl sulfanilamide (septazine, Goissedet et al,[6] 1936).

The second group consists of those derivatives in which substitutions have been made in the sulfonamide group at the other end of the benzene ring leaving the para-amino group free. Among these may be mentioned sulfanilyl sulfanilamide (Rosenthal et al,[7] 1937) and its dimethyl derivative (Domagk,[8] 1937), diamino-diphenyl sulphone (Buttle et al,[9] 1937), sulfapyridine (Whitby,[10] 1938), and sulfathiazole (Fosbinder and Walter,[11] 1939) and its methyl derivative sulfamethylthiazole (Herrell and Brown,[12] 1939) recently introduced and now being submitted to experimental investigation in the laboratory and the clinic.

The reason for grouping the sulfonamide derivatives in this fashion, which will, perhaps, become more evident as I proceed, is because it would appear to bear at least some relation to breadth and effectiveness as well as mode of therapeutic action.

Comparative studies in experimental animals on the protective action of the sulfonamide group of drugs, and *in vitro* observations on their bacteriostatic and bactericidal properties, beginning with the original observations of Domagk[3] on the curative action of prontosil on β-hemolytic streptococcal infection in mice down to the most recent observations on sulfamethylthiazole[12] on staphylococcal infections have covered

a wide range of bacteria and viruses. At first glance the results reported by various investigators often appear conflicting, no doubt in large part due to numerous variables which inevitably occur in experiments of this kind before an acceptable, standard technique has been evolved.

In spite of these difficulties certain high-lights stand out, which may serve to bring some degree of order out of the apparent confusion, even though many points still remain at issue.

The first significant experimental observations were, of course, those of Domagk,[3,5] that the azo dyes, prontosil and neoprontosil, though exerting no demonstrable bactericidal or bacteriostatic action *in vitro*, were nevertheless highly effective *in vivo* against virulent hemolytic streptococci, 100 per cent of treated mice surviving 10 m.l.d. for seven days in his first recorded experiment. These observations have been amply confirmed[13] by numerous subsequent observers, though in general without quite such perfect results.

The second highly important contributions were those of the Tréfouëls[14] and their collaborators, who showed that sulfanilamide by itself without the azo linkage was not only effective against hemolytic streptococcal infections in mice but also possessed active bacteriostatic properties *in vitro*. These observations provided the basis for the now widely accepted view that the azo dyes, prontosil and neoprontosil, are changed in the animal body and owe at least the major portion of their activity to the liberation of sulfanilamide, which is the active component, a view well supported by the studies of Colebrook, Buttle and O'Meara,[15] Fuller,[16] and Long and his collaborators.[17]

If this be correct, sulfanilamide weight for weight should be more effective as a chemotherapeutic agent than prontosil, neoprontosil and benzyl-sulfanilamide, provided variations in absorption are not so great as to render comparisons invalid. Though the pitfalls in such comparisons are numerous and there is not complete agreement, I believe it safe to state that the majority of workers have recorded experiments indicating that sulfanilamide, weight for weight, is at least equivalent to or superior to the prontosils and benzyl compounds, so far as chemotherapeutic effectiveness against hemolytic streptococci is concerned. Not only does this appear to be so with hemolytic streptococci but also in the case of many other bacteria. Among them may be included meningococci, pneumococci, staphylococci, Friedländer's bacillus and brucella.

If we turn now to the other group of compounds in which substitu-

tions in the sulfonamide group have been made, the picture appears quite different. Here we find compounds which are at least equivalent to, if not superior to, sulfanilamide in experimental streptococcal and meningococcal infections. At the same time the scope of their effectiveness appears to be wider. Conspicuous among these is sulfapyridine, the superiority of which in pneumococcal infections is already well established. Animal experiments, furthermore, suggest that it is more effective than sulfanilamide in staphylococcal[18] and Friedländer's bacillus[13] infections. Two newcomers, still in the stage of experimental investigation, are sulfathiazole and sulfamethylthiazole. In preliminary studies by McKee, Rake, Greep and van Dyke,[19] sulfathiazole would appear at least equivalent to sulfapyridine, so far as hemolytic streptococci, pneumococci, and meningococci are concerned, while sulfamethylthiazole may perhaps be even more effective in staphylococcal infections,[12] though further data are necessary to confirm this.

Although comparative experiments would appear to suggest that the differences in scope and effectiveness exhibited by these two groups of sulfonamide derivatives are largely quantitative, this cannot be accepted as proved at present because standard methods for comparative assay have not been used. As emphasized by Marshall,[20] strains of organisms, size of inoculum, amount, spacing and method of dosage, duration of therapy and period of observation have varied so much from worker to worker that accurate quantitative comparison is impossible with active compounds having different ratios of absorption and excretion.

Whether the differences exhibited indicate actual qualitative differences in specificity for different organisms is likewise impossible to answer with certainty at present. The much greater effectiveness of sulfapyridine against the pneumococcus, for example, would suggest specificity, but since there is some evidence that it is also more effective against hemolytic streptococci,[10,18,18] staphylococci[13,18] and Friedländer's bacilli,[13] this may be merely a quantitative difference.

The relative importance of the amino group and the sulfonamide group in determining chemotherapeutic activity likewise cannot be stated dogmatically at present. Active compounds, with rare and somewhat dubious exceptions, either have a free amino group in the para-position to the sulfonamide group or a substituted group (nitro group or azo linkage) which is readily changed to an amino group in the animal body. Substitutions which do not permit change to an amino group,

e.g., acetyl sulfanilamide, in general, appear to be inactive.

Since, by definition the group of compounds under discussion contain sulfur in the sulfonamide group it is irrelevant to enter into a discussion here as to whether sulfur is an essential component for chemotherapeutic activity, though it may be remarked in passing that this is unlikely (Rosenthal, Bauer and Elvove[21]).

Whatever the ultimate solution of the foregoing problems may be, the clinical significance of these comparative *in vivo* and *in vitro* studies of the sulfonamide derivatives against various bacteria is obvious, since they point the way to the selection of the most effective drugs among the various compounds available. Furthermore, they suggest at least that the road to further improvement in scope and effectiveness of action lies along the way of substitutions in the sulfonamide group.

It hardly need be pointed out, however, that other factors besides relative chemotherapeutic efficiency in mice and bacteriostatic activity in the test tube may of necessity modify the selection of the most suitable drug for practical therapeutics in man, such factors as primary toxicity, absorbability, and human idiosyncrasy to untoward reactions, to mention a few.

For example, the experimental studies indicate clearly the superiority of sulfapyridine over sulfanilamide in pneumococcal infections, an observation which has found ample confirmation in the clinic. In hemolytic streptococcal infections on the other hand, the fact that sulfapyridine seems to possess some superiority in laboratory studies, would hardly justify at present the complete abandonment of sulfanilamide in favor of sulfapyridine, in view of the poorer absorbability, greater variability in acetylation and greater tendency to induce untoward reactions, such as nausea, vomiting and hematuria, exhibited by sulfapyridine. In the end, of course, carefully controlled studies in patients, so well illustrated by the studies of the last year on sulfapyridine in lobar pneumonia, must determine the issue in favor of this or that compound in any given infection.

It is now so well established by the work of numerous investigators that the therapeutic activity of the sulfonamide compounds is dependent upon their bacteriostatic action, rather than upon a capacity to stimulate the defensive mechanisms of the host, that it seems hardly necessary in this discussion to marshal the evidence in support of this view. Nor does it seem profitable to enter into a discussion of the several theories

concerning the mechanism by which bacteriostasis is brought about, since none of them has as yet attained the position of final proof.

A few points with clinical implications, however, emerge from the welter of *in vitro* and *in vivo* studies on mechanism of action which may be briefly touched upon.

The failure of the azo dyes to exhibit bacteriostatic action *in vitro* as contrasted with sulfanilamide and those derivatives in which substitution has been made in the sulfonamide group has already been mentioned and its implications pointed out.

In vitro experiments, principally on hemolytic streptococci but also on other bacteria, with concentrations of sulfanilamide equivalent to those attained in the blood of patients and with a suitably small initial inoculum, have commonly resulted after an initial lag period in variable degrees of bacteriostasis without true bactericidal effect. In the presence of whole blood, as contrasted with blood deprived of leukocytes, prompt killing of streptococci occurs. Similarly a much greater bacteriostatic action and frequently a bactericidal action in the presence of whole blood has been found in our laboratory by Dr. Haury with sulfapyridine and staphylococci. These observations have strongly suggested that coöperative activity on the part of phagocytic and perhaps other immunity mechanisms of the host is important or essential in the final recovery of patients being treated with the sulfonamide drugs. Numerous animal experiments, among others those of Gay and Clark[22] in streptococcal infection in rabbits and Menefee and Poston[23] on brucella infections in guinea pigs, strongly support this view. When taken in conjunction with the oft repeated observation, particularly in streptococcal and staphylococcal infections, that relapse of infection with ultimate death often occurs in a considerable proportion of experimental animals following cessation of treatment, this fact assumes a position of significant importance in human therapeutics, particularly in those infections which do not naturally run a relatively brief, self-limited course to recovery.

To cite specific examples, facial erysipelas in adults in general runs a relatively short, self-limited course. It responds dramatically to sulfanilamide therapy and relapses are exceedingly rare even though therapy be discontinued relatively soon after the temperature has fallen to normal. By and large the same may be said to be true in lobar pneumonia treated with sulfapyridine, provided treatment is carried till the 7th to 9th day from onset, at which time the immunity mechanism commonly

comes into operation. Erysipelas of the new-born, on the other hand, usually runs a progressive course with generalization of the infection and ultimate death. Likewise responding brilliantly to early treatment, relapse is apt to occur with early cessation of treatment.

Streptococcal sinusitis, mastoiditis and sepsis, gonococcal prostatitis and staphylococcal pyemia provide other examples in which initial response to chemotherapy may give a false sense of security, masking the fact that infection is still smoldering in local suppurative foci, ready to flare up when chemotherapy is discontinued, a problem ably discussed recently by Converse[24] in the case of otitic infections.

Obviously, the problem of combined chemo and serum therapy versus chemotherapy alone in pneumococcal, meningococcal, and gas bacillus infections as well as others, is brought sharply into focus by the laboratory studies which indicate the coöperative role of immunity mechanisms in fortifying the chemotherapeutic action of the sulfonamide derivatives, but lack of time and the sparsity of adequately controlled comparative studies in man induce me to pass it by without further discussion. Nor shall I comment on the important observations of White and Parker[25] on the influence of temperature and those of Lockwood[26] on the influence of nutritional environment on the bacteriostatic action of sulfanilamide, since their possible clinical significance is not yet clearly apparent, though the latter, as suggested by Lockwood, may have an important bearing on the tendency for bacteria to survive in walled off suppurative lesions unless these be surgically drained.

Pharmacological studies, conspicuously those of Marshall[20] and his collaborators, have likewise contributed much of value to the practical application of chemotherapy in man. These studies have been concerned particularly with problems of toxicity, absorption, distribution in the body, conjugation to the inactive acetyl derivatives, and excretion. They have had an especially useful bearing on problems of amount and spacing of dosage in relation to establishment and maintenance of adequate blood levels. The development of Marshall's method[27] for the quantitation of blood concentrations of total and free sulfonamide compounds has provided not only a useful guide to dosage but has made it possible to acquire information concerning the relation of blood levels to therapeutic effect and untoward reactions in patients under treatment.

Comparative observations on blood concentrations following a single dose *per os* of 0.1 gm./K in dogs indicate a considerably more rapid

absorption of sulfanilamide than of sulfapyridine or sulfathiazole. At the same time the percentage of administered drug recovered in the urine during the ensuing twenty-four hours indicates a much more complete absorption and a somewhat more rapid rate of diffusion and excretion for the former. These observations find their counterpart in clinical observations showing the range of blood concentrations of sulfanilamide[27] and sulfapyridine[28] and a few recent observations on sulfathiazole[29] four hours after an initial dose *per os*. They indicate the greater urgency for an initial intravenous treatment with sulfapyridine than is the case with sulfanilamide in critically ill patients in whom it is desirable to establish an adequate concentration in the blood and body fluids as rapidly as possible.

Furthermore, the more rapid rate of excretion in the case of sulfanilamide suggests that treatment every four hours, day and night, is perhaps more essential for the maintenance of continuously adequate blood levels with this drug than may be the case with sulfapyridine. At least it has been our experience that treatment at six hour intervals with sulfapyridine will, in many instances, maintain an adequate blood level, once established, and even at eight to twelve hour intervals under parenteral treatment[30] by hypodermoclysis or intravenously. The problem is complicated in man, by so much individual variation from patient to patient that wider spacing of dosage with sulfapyridine than the customary four hour interval cannot at present be advocated as a routine but should be resorted to only when the presence of the higher range of blood levels indicates that it is permissible in a particular individual.

Pharmacological observations on the conjugation of sulfanilamide in animals have shown so much species difference in the capacity to convert sulfanilamide to the inactive acetyl compound that it has been found necessary to study this problem in man. Marshall[27] found that 10 to 20 per cent of the total sulfanilamide in the circulating blood was in the conjugated form and Stewart, Rourke and Allen[31] have shown that the ratio between free and acetyl sulfanilamide varies considerably from individual to individual. In the case of sulfanilamide, however, the degree of acetylation in man and individual variation would not appear to be of sufficient magnitude to be of clinical import and in practical therapeutics may be disregarded. A few preliminary observations[29] in patients treated with sulfathiazole and sulfamethylthiazole suggest that the same may hold for these compounds, but more extensive studies are

necessary before the possible range of individual variation will be known.

With sulfapyridine, on the other hand, the picture is quite different. The degree of acetylation may vary unpredictably over a wide range from patient to patient.[30,32] Recognition of this fact is of great importance in therapeutics if blood levels are to be used as a guide to amount and spacing of dosage, for a low or falling concentration of free sulfapyridine may be mistakenly regarded as an indication for increasing dosage when, in fact, the total concentration is rising with an increasing proportion of acetyl sulfapyridine present. On more than one occasion I have seen this mistake made, to be followed by gross hematuria, colic, temporary anuria and azotemia.

I have used the phrase adequate blood concentration without specifying what that may be, and I must confess to some degree of uncertainty concerning this, an uncertainty which has arisen from the now well established and perhaps unexpected finding that many cases of pneumococcal lobar pneumonia respond as promptly and dramatically to sulfapyridine with blood levels of free sulfapyridine under 5 mg. per cent as do those with higher levels.[30] Quite early in the use of sulfanilamide in hemolytic streptococcal infections, 10 mg. per cent or thereabouts was somewhat arbitrarily advanced as a desirable and necessary blood concentration for satisfactory therapeutic results and there can be no doubt that experience has shown that blood concentrations at this level are effective. Since adequate comparative data on the possible effectiveness of lower blood concentrations are not available, it is of no value to discuss the point, further than to suggest that it might be desirable to reëxamine this question in the light of the results with sulfapyridine in pneumococcal pneumonia.

I have purposely not touched upon the general problems of toxicity and untoward reactions since they are to be discussed by a subsequent speaker on this program. If I have disappointed you by using the subjunctive more than you would like, I can only say that it has seemed to me the part of wisdom to be neither too positive nor too dogmatic in our present state of fragmentary, though rapidly increasing knowledge concerning the general principles involved in chemotherapy with the sulfonamide derivatives. That the future of chemotherapy will witness advances equal to or more brilliant than those of the last few years, there seems little doubt.

REFERENCES

1. Foerster. Sepsis im Anschluss an ausgedehnte Periporitis; Heilung durch Streptozon, *Zentralbl. f. Haut u. Geschlechtskr.*, 1933, *45:* 549.
2. Gelmo, P. Über Sulfamide der p-Amidobenzolsulfonsäure, *J. prakt. Chem.*, 1908, *77:* 369.
3. Domagk, G. Ein Beitrag zur Chemotherapie der bakteriellen Infektionen, *Deutsche med. Wchnschr.*, 1935, *61:* 250.
4. Mietzsch, F. and Klarer, J. [Title not available.] *Deutsches Reichpatent*, 1932, *607:* 537.
5. Domagk, G. Chemotherapie der bakteriellen Infektionen, *Ang. Chem.*, 1935, *48:* 657.
6. Goissedet, P. *et al.* De l'action du radical sulfamide: SO_2NH_2 sur l'infection streptococcique expérimentale, *Compt. rend. Soc. de biol.*, 1936, *121:* 1082.
7. Rosenthal, S. M., Bauer, H. and Branham, S. E. Studies in chemotherapy; comparative studies in sulphonamide compounds in experimental pneumococcus, streptococcus and meningococcus infections, *Pub. Health Rep.*, 1937, *52:* 662.
   Bauer, H. and Rosenthal, S. M. Studies in chemotherapy; some new sulphur compounds active against bacterial infection, *ibid.*, 1938, *53:* 40.
8. Domagk, G. Weitere Untersuchungen über die chemotherapeutische Wirkung sulfonamidhaltiger Verbindungen bei bakteriellen Infektionen, *Klin. Wchnschr.*, 1937, *16:* 1412.
9. Buttle, G. A. H. *et al.* Treatment of streptococcal infections in mice with 4:4′ diaminodiphenylsulphone, *Lancet*, 1937, *1:* 1331.
10. Whitby, L. E. H. Chemotherapy of pneumococcal and other infections with 2-(*p*-aminobenzenesulphonamide) pyridine, *Lancet*, 1938, *1:* 1210.
11. Fosbinder, R. J. and Walter, L. A. Sulfanilamide derivatives of heterocyclic amines, *J. Am. Chem. Soc.*, 1939, *61:* 2032.
12. Herrell, W. E. and Brown, A. E. The clinical use of sulfamethylthiazol in infections caused by Staphylococcus aureus, *Proc. Staff Meet. Mayo Clin.*, 1939, *14:* 753; also Winthrop Chemical Co., Inc., *Unpublished studies.*
13. Long, P. H. and Bliss, E. A. *The clinical and experimental use of sulfanilamide, sulfapyridine and allied compounds.* New York, Macmillan, 1939.
14. Tréfouël, J., Tréfouël, Mme. J., Nitti, F. and Bovet, D. Activité du p-amidophénylsulfamide sur les infections streptococciques expérimentales de la souris et du lapin, *Compt. rend. Soc. de biol.*, 1935, *120:* 756.
    Fourneau, E., Tréfouël, J., Tréfouël, Mme. J., Nitti, F. and Bovet, D. Chimiothérapie de l'infection pneumococcique par la di- (p-acétylaminophényl) sulfone, *Compt. rend. Acad. de sc.*, 1937, *205:* 299.
15. Colebrook, L., Buttle, G. A. H. and O'Meara, R. A. Q. Mode of action of *p*-aminobenzenesulphonamide and prontosil in hemolytic streptococcal infections, *Lancet*, 1936, *2:* 1323.
16. Fuller, A. T. Is *p*-aminobenzenesulphonamide the active agent in prontosil therapy? *Lancet*, 1937, *1:* 194.
17. Long, P. H. and Bliss, E. A. Para-amino-benzene-sulfonamide and its derivatives; experimental and clinical observations on their use in hemolytic streptococcic infection, *J.A.M.A.*, 1937, *108:* 32.
    Feinstone, W. H., Bliss, E. A., Ott, E. and Long, P. H. Observations concerning toxicity, absorption and therapeutic effect of sulphanilamide and certain related organic sulphur-containing compounds, *Bull. Johns Hopkins Hosp.*, 1938, *62:* 565.
18. Whitby, L. E. H. Chemotherapy of bacterial infections, *Lancet*, 1938, *2:* 1095.
19. McKee, C. M., Rake, G., Greep, R. O. and van Dyke, H. B. Therapeutic effect of sulfathiazole and sulfapyridine, *Proc. Soc. Exper. Biol. & Med.*, 1939, *42:* 417.
20. Marshall, E. K., Jr. Bacterial chemotherapy; the pharmacology of sulfanilamide, *Physiol. Reviews*, 1939, *19:* 240.
21. Rosenthal, S. M., Bauer, H. and Elvove, E. Studies in chemotherapy: antibac-

terial action of some aromatic arsenic, sulfur, and nitro compounds, *Pub. Health Rep.*, 1939, *54:* 1317.

22. Gay, F. P. and Clark, A. R. On mode of action of sulfanilamide in experimental streptococcus empyema, *J. Exper. Med.*, 1937, *66:* 535.
23. Menefee, E. E., Jr. and Poston, M. A. Effects of sulfanilamide on Brucella melitensis *var.* melitensis, abortus and suis, *J. Bacteriol.*, 1939, *37:* 269.
24. Converse, J. M. Recurrence of otitic infections due to the beta-hemolytic streptococcus, *J.A.M.A.*, 1939, *113:* 1383.
25. White, H. J. and Parker, J. M. The bactericidal effect of sulfanilamide upon beta hemolytic streptococci in vitro, *J. Bacteriol.*, 1938, *36:* 481.
26. Lockwood, J. S. Studies on the mechanism of the action of sulfanilamide, *J. Immunol.*, 1938, *35:* 155.
27. Marshall, E. K., Jr., Emerson, K., Jr. and Cutting, W. C. Para-aminobenzenesulfonamide; absorption and excretion; method of determination in urine and blood, *J.A.M.A.*, 1937, *108:* 953.
28. Long, P. H. and Feinstone, W. H. Observations upon the absorption and excretion of sulfapyridine (2 sulfanilyl aminopyridine), *Proc. Soc. Exper. Biol. & Med.*, 1938-39, *39:* 486.
29. Blake, F. G. and Sadusk, J. F., Jr. *Unpublished studies.*
30. Blake, F. G. and Haviland, J. W. Sulfapyridine in pneumococcal, streptococcal and staphylococcal infections, *Internat. Clin.*, 1939, new ser. *4:* 1.
31. Stewart, J. D., Rourke, J. W. and Allen, J. G. Excretion of sulfanilamide, *J.A.M.A.*, 1938, *110:* 1885.
32. Stokinger, H. E. Absorption, acetylation and excretion of 2 sulfanilamide pyridine (Dagenan, M & B 693), *Proc. Soc. Exper. Biol. & Med.*, 1939, *40:* 61.